



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/47, 16/18, G01N 33/564	A2	(11) International Publication Number: WO 98/22503 (43) International Publication Date: 28 May 1998 (28.05.98)
(21) International Application Number: PCT/NL97/00624 (22) International Filing Date: 14 November 1997 (14.11.97) (30) Priority Data: 1004539 15 November 1996 (15.11.96) NL (71) Applicants (for all designated States except US): STICHTING SCHEIKUNDIG ONDERZOEK NEDERLAND [NL/NL]; Laan van Nieuw Oost-Indië 131, NL-2593 BM Den Haag (NL). STICHTING VOOR DE TECHNISCHE WETENSCHAPPEN [NL/NL]; Raadstede 15/19, NL-3431 HA Nieuwegein (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): VAN VENROOIJ, Waltherus, Jacobus, Wilhelmus [NL/NL]; Eleonoraweg 16, NL-6523 MZ Nijmegen (NL). SCHELLEKENS, Gerardus, Antonius [NL/NL]; Zwanenveld 37-03, NL-6538 XV Nijmegen (NL). RAATS, Jozef, Maria, Hendrik [NL/NL]; Van Diemberbroeckstraat 65, NL-6512 BA Nijmegen (NL). HOET, René, Michael, Antonius [NL/NL]; Weezenhof 35 - 40, NL-6536 HB Nijmegen (NL).		(74) Agent: ALTENBURG, Bernardus, Stephanus, Franciscus; Octrooibureau Los en Stigter B.V., Weteringschans 96, NL-1017 XS Amsterdam (NL). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>In English translation (filed in Dutch).</i> <i>Without international search report and to be republished upon receipt of that report.</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13bis separately from the description.</i> <i>Date of receipt by the International Bureau:</i> 05 December 1997 (05.12.1997)
(54) Title: PEPTIDE DERIVED FROM AN ANTIGEN RECOGNIZED BY AUTOANTIBODIES FROM PATIENTS WITH RHEUMATOID ARTHRITIS, ANTIBODY DIRECTED AGAINST SAID PEPTIDE, A COMBINATORIAL ANTIGEN, AND A METHOD OF DETECTING AUTO-IMMUNE ANTIBODIES (57) Abstract <p>The invention relates to a peptide derived from an antigen recognized by autoantibodies, which peptide is reactive with autoimmune antibodies from a patient suffering from rheumatoid arthritis. The peptide according to the invention possesses a modified arginine residue. The invention also relates to antibodies against the peptide and a method of detecting autoimmune antibodies.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MV	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NI	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakistan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Peptide derived from an antigen recognized by auto-antibodies from patients with rheumatoid arthritis, antibody directed against said peptide, a combinatorial antigen, and a method of detecting auto-immune antibodies

The present invention relates to a peptide derived from an antigen recognized by autoantibodies from patients with rheumatoid arthritis, which peptide is reactive with autoimmune antibodies from a patient suffering from rheumatoid arthritis.

Such a peptide is known from the European patent application 0 511 116 (Clonatec S.A.). This application describes an antigen comprising a filaggrin or pro-filaggrin fragment. The peptide is recognized by rheumatoid arthritis-specific autoimmune antibodies. Rheumatoid arthritis (RA) is a systemic autoimmune disease. It is the most commonly occurring inflammatory disease of the joints, it is chronic and may lead to severe physical disablement.

The object of the present is to provide a peptide which is reactive with autoimmune antibodies from a patient suffering from rheumatoid arthritis, which peptide is suitable for diagnostic research with increased specificity while also being useful for other purposes such as obtaining (raising, selecting and isolating) poly- and monoclonal antibodies.

To this end the peptide according to the invention is characterized in that the derived peptide that is reactive with autoimmune antibodies, corresponds to a part of a mRNA molecule coding for the antigen, said part comprising a codon for an arginine residue, and the arginine residue in the derived peptide, which is reactive with autoimmune antibodies, is a modified arginine residue.

Surprisingly, the peptide according to the invention that possesses a modified arginine residue, proved to be very suitable for the specific diagnosis of rheumatoid arthritis.

To this day, no specific serological test is available for RA. The only test frequently employed is based on the determination of rheumatoid factors (RF; Ref. 1) which are found in 70% of the RA patients. However, this test is not very specific and is characterized by a relatively large number of false positives. For patients suffering from systemic lupus erythematosus the percentage of false positives is approximately 20% and for healthy individuals approximately 5%.

Preferably the peptide is characterized in that the modified arginine residue's side chain is a side chain according to Formula I on the formula sheet, in which

$X = \text{NH}_2, \text{CH}_3, \text{NHCH}_3 \text{ or } \text{N}(\text{CH}_3)_2;$

$Y = \text{O}, \text{NH}, \text{NHCH}_3 \text{ or } \text{N}(\text{CH}_3)_2;$

$Z = \text{O}, \text{NH} \text{ or } \text{CH}_2; \text{ and}$

$n = 2, 3 \text{ or } 4,$ on the condition that when $X = \text{NH}_2$ and $Z = \text{NH}, Y$ is not NH ; and the modified arginine residue is in particular a citrulline residue. For citrulline, $X = \text{NH}_2, Y = \text{O}, Z = \text{NH}$ and $n = 3$.

A preferred peptide is the peptide selected from the group of peptides having the Formula II - X on the formula sheet.

By using the peptide according to Formula II, it is possible to establish the presence of rheumatoid arthritis in about 36% of patients actually suffering from rheumatoid arthritis, while the percentage of false positives for other autoimmune diseases and healthy individuals is less than 2%.

According to a favourable embodiment the peptide is a cyclic peptide, for instance, due to the presence of a cystine residue.

In some cases such a cyclic peptide exhibits an increased immunological affinity.

The preferred cyclic peptide is the peptide having the Formula XI on the formula sheet.

Preferably the peptide is a synthetic peptide.

The reactive peptide according to the invention can be obtained pure and in large quantities by means of

organic synthesis, making immunological testing on a large scale possible.

According to an alternative embodiment, the peptide in accordance with the invention is characterized in that the peptide is obtained by the proteolytic treatment of (pro)filaggrin, separation of peptide fragments formed by proteolysis and subsequent selection on the presence of a modified arginine residue in a peptide which was formed during the proteolytic treatment.

In this manner peptides can be identified which can increase the sensitivity of a rheumatoid arthritis test. The term sensitivity is in the present application to be understood to mean the ability of a test to properly identify a patient suffering from rheumatoid arthritis.

According to a favourable embodiment, the antigen is (pro)filaggrin, and the peptide is reactive with a rheumatoid arthritis patient's autoimmune antibodies which are reactive with (pro)filaggrin.

The peptide has been shown to be very suitable for high-specificity testing (few false positives) for rheumatism.

The present invention also relates to an antibody which is cross-reactive with an antibody raised against a peptide according to the invention.

Such an antibody is useful for the indication of rheumatoid arthritis by analysing sections of biopsy samples and immunological tests of the sandwich type.

The antibody is preferably a monoclonal antibody.

According to another preferred embodiment, the antibody is obtained by using as antigen a peptide in accordance with the invention.

A suitable antibody according to the invention is characterized in that it is cross-reactive with the antibody as produced by Escherichia coli TG1 with plasmid RA3, deposited at the Centraalbureau voor Schimmelcultures, at Baarn, the Netherlands under accession number CBS143.96.

The invention further relates to an organic compound comprising a part that is able to compete with a peptide according to one of the claims 1 to 9 for binding

to an antibody which is specific for said peptide, wherein at least said part of the organic compound can be prepared by means of combinatory chemistry.

Such organic compounds are found by competitive
5 selection wherein a peptide of the invention competes for recognition by an antibody of the invention, such as the antibody produced by E. coli. CBS143.96. The organic compounds, which are often cheaper to produce than antigens that are prepared solely on the basis of amino acids that
10 may or may not comprise side chains, are suitable for immunological kits for diagnosing RA. Also, after coupling to a solid carrier, said organic compounds could be applied to lower, through adsorption, the level of autoimmune antibodies in the blood of patients suffering from
15 RA.

Finally, the invention relates to a method of detecting autoimmune antibodies against rheumatoid arthritis.

The method according to the invention is characterized in that in an immunological test at least one
20 immunologically active molecule selected from the group consisting of i) a peptide according to the invention; ii) a recombinatory organic molecule according to the invention; and iii) an antibody according to the invention is
25 used.

In addition to increased sensitivity other advantages are achieved, in particular better reproducibility, quantitative information and better applicability for prognostic purposes.

30 To a person skilled in the art it will be apparent that there are a number of possible variations to the present invention as specified by the appended claims. For instance, the peptides mentioned on the formula sheet may also be part of other oligopeptides. They may be provided
35 at one or both ends with one or more other amino acids while also, two or more peptides according to the invention may be part of one oligopeptide. It is also possible to shorten the peptides by one or more amino acids, provided this does not have a significantly adverse effect on

the reactivity. The expert is familiar with the manner in which peptides and organic compounds according to the invention may optionally be labelled or be coupled to a carrier, and how on the basis of such antigens an immunological test may be developed, using the standard techniques well-known in the field.

The invention will now be explained in more detail by means of the following example.

10 Materials and methods

Peptide synthesis: Peptides were selected for synthesis on the basis of amino acid sequences derived from known cDNA sequences of human profilaggrin (Ref. 2; Ref. 3). The peptides were synthesized on solid phase using the method described by Schellekens et al. (Ref. 4). The peptides were at least 95% pure, as determined by the elution profile by means of reversed phase chromatography and the relative absorption at 214 nm. The composition of the peptides was confirmed by means of mass spectrometry (MALDI-MS). All peptides were synthesized as peptide amides.

TABLE 1

Synthesized peptides

The peptide names starting with "cf" are based on the C-terminal end (amino acids 306-324); and the peptide names starting with "nf" are based on the sequence near the N-terminal end (amino acids 18-32 for nfc1).

Amino acid sequences based on cDNA of a profilaggrin repeat.

Name	Peptide sequence*
cfc1	S H Q E S T <u>X</u> G R S R G R S G R S G S
cfc2	S H Q E S T R G <u>X</u> S R G R S G R S G S
cfc3	S H Q E S T R G R S <u>X</u> G R S G R S G S
cfc4	S H Q E S T R G R S R G <u>X</u> S G R S G S
cfc5	S H Q E S T R G R S R G R S G <u>X</u> S G S
cfc6	S H Q E S T <u>X</u> G <u>X</u> S R G R S G R S G S
cfc7	S H Q E S T <u>X</u> G R S <u>X</u> G R S G R S G S
cfc8	S H Q E S T <u>X</u> G R S R G <u>X</u> S G R S G S
cfc9	S H Q E S T <u>X</u> G R S R G R S G <u>X</u> S G S
cf	S H Q E S T R G R S R G R S G R S G S
cfA	S H Q E S T A G R S R G R S G R S G S
cfE	S H Q E S T E G R S R G R S G R S G S
cfQ	S H Q E S T Q G R S R G R S G R S G S
nfc1	T G P S T R G R Q G S <u>X</u> H E
nf	E S S H G W T G P S T R G R Q G S R H E

* (A = alanine; G = glycine; H = histidine; E = glutamic acid; P = proline; R = arginine; Q = glutamine; S = serine; T = threonine; W = tryptophan; X = citrulline)

Detection by means of ELISA

Via an N-oxysuccinimide surface the peptides were covalently coupled to the wells of 96-well microtitre plates (Costar amide binding plates) in an amount of 1 $\mu\text{g/well}$. Coupling took place for 16 hours at 4°C and pH 9.0. The plates were blocked for 1 hour with 2% bovine serum albumin. The sera were diluted 200 times in a diluent (0.3% BSA, 350 mM NaCl, 10 mM Tris-HCl pH 7.6, 1% vol./vol. Triton X-100, 0.5% w./vol. Na-deoxycholate, 0.1% SDS) supplemented with 10% normal rabbit serum, and incubated for one hour at room temperature. After washing the plates (3 times with PBS containing 0.05% by vol. of Tween®20), 100 μl of antihuman IgG conjugated with peroxidase (Dako P214), 1000 times diluted in dilution buffer, was added to the wells. After incubation for 1 hour at room temperature, the plates were washed 3 times with PBS/Tween®, and bound antibodies were detected with tetramethyl benzidine as a substrate. After 10 minutes the reaction was stopped by adding 100 μl of 2 M sulphuric acid per well. Readout occurred at 450 nm. Sera having an OD_{450} of 0.2, after deduction of the blank for the respective serum (a well without a coupled peptide), were considered to be positive.

Results

The results are listed in Table 2. In total, 288 sera from patients suffering from rheumatoid diseases were used, 132 of which were from patients suffering from rheumatoid arthritis.

TABLE 2

Results with peptide cfc1 to cfc9 (Formula II to X of the formula sheet)

5

	Peptide*	RA sera		control				
		(%)		sera ¹	SLE ²	SSC ³	pSS ⁴	PM/DM ⁵
		(n =		(%)	(%)	(%)	(%)	(%)
		134)		(n =	(n =	(n =	(n =	(n =
				154)	50)	50)	50)	50)
	cfc1	49	(36)	1	(0.6)	1	(2)	0
10	cfc2	27	(20)	4	(2.6)	1	(2)	1
	cfc3	37	(28)	2	(0.6)	0		1
	cfc4	32	(24)	2	(1.3)	0		0
	cfc5	64	(48)	1	(0.6)	0	1	(2)
	cfc6	65	(48)	1	(0.6)	0	2	(4)
15	cfc7	60	(45)	1	(0.6)	0	1	(2)
	cfc8	55	(41)	1	(0.6)	0	1	(2)
	cfc9	57	(42)	1	(0.6)	0	2	(4)

20

1) Control sera are from patients suffering from rheumatic diseases other than RA.

2) SLE is systemic lupus erythematosus.

3) pSS is primary Sjögren's syndrome.

4) SSC is systemic scleroderma.

5) PM/DM is polymyositis/dermatomyositis.

25

Of the total of 134 RA sera from patients suffering from rheumatoid arthritis, 102 were positive with at least one peptide from the cfc1 to cfc9 series. Therefore, when using these peptides, the sensitivity was 76% (102/134).

30

Of the total of 354 control sera, 13 sera were positive on at least one peptide from the cfc1 to cfc9 series. Therefore the test sensitivity, expressed as percentage of true

positives, was 96%. Of the 37 sera that were reactive with cfc3, none were not recognized by peptide cfc1 or cfc23. Of the sera that were reactive with cfc7, cfc8 and cfc9, none were not recognized by cfc1, cfc2, cfc4, cfc5 or cfc6. This means that cfc2, cfc7, cfc8 and cfc9 do not contribute to the test sensitivity and a test sensitivity of 76% may be realized by using the combination of the peptides cfc1, cfc2, cfc4, cfc5 and cfc6.

It should be noted that these percentages depend on the specificity-threshold value applied by applicants. The same data (from the ELISA experiments) can be interpreted as a sensitivity of approximately 80-85% by choosing a slightly lower sensitivity, which incidentally, is still much better than the one obtainable when using the known rheumatoid factor test (Ref. 1).

Sera from patients suffering from various infectious diseases (Borrelia, syphilis, malaria, endocarditis, Legionella, tuberculosis, mycoplasma, Yersinia, salmonella, parvovirus B19, Epstein-Barr virus, rubella, schistosomiasis, Toxoplasma, leishmaniasis, Chagas' disease) were tested for the presence of antibodies reactive with cfc1. Of the 308 tested sera 9 were positive. This means that the specificity was 97%, a considerable improvement compared with the RF test.

Variants of cfc1 wherein citrulline was replaced by a neutral (alanine; cfA), acid (glutamic acid; cfE) or amide (glutamine; cfQ) residue, did not seem to be immunologically reactive. The same applies to the control peptide cf, which does not possess a modified arginine residue.

With the aid of the above-described ELISA, a cyclic variant (with the Formula XI on the formula sheet, in which two cysteine residues (C) are bound by means of a sulphur bridge) of cfc1 was tested for 134 RA sera. This cyclic variant was shown to be reactive with 85 sera (63%), signifying an increase in sensitivity. Of the 154 sera of patients suffering from rheumatic diseases other than RA, 3 were shown to be positive (specificity 98%). The priority document of the present application reports 5

falsely determined positives. However, it has been shown that in two of these cases the patients did indeed suffer from RA. Not one serum from 59 healthy individuals was positive with this cyclic peptide, nor with any of the peptides cfc1 to cfc9. The cyclic peptide variant was shown to be reactive with 4 sera of the 200 additional control sera (50 SLE, 50 SCC, 50 pSS, 50 PM/DM) so that the specificity in respect of these sera was 98%. Of the sera from patients suffering from various infectious diseases (308 sera as described above), 7 sera were shown to be positive with the cyclic peptide variant so that in this case also the specificity in respect of these sera was 98%. The use of the cyclic peptide variant thus enhances the sensitivity compared with the individual linear peptide variants, but the specificity is also enhanced due to an improved signal/noise ratio in the described ELISA test.

A second citrulline-substituted peptide (nfc1) was shown to be specifically reactive with 10% of the RA sera, but not with the control peptide nf, which does not comprise citrulline. Of the RA sera reactive with nfc1, some were not reactive with cfc1 to cfc9. Therefore, it is possible to increase the sensitivity of a test for rheumatoid arthritis by applying more peptides comprising a modified arginine residue.

Obviously, a peptide may comprise several modified arginine residues, but the peptide may also comprise one or more non-modified arginine residues.

Applicants believe that modified amino acids, in particular those derived from arginine residues, could possibly also play a role in other autoimmune diseases. For this reason, the invention is also aimed at peptides comprising modified amino acids that are reactive with auto-antibodies from patients suffering from autoimmune diseases other than RA. This relates especially to peptides comprising a modified arginine residue wherein $X = \text{NHCH}_3$ (wherein $Y = \text{NH}$ or NCH_3) or $\text{NH}(\text{CH}_3)_2$ is, which peptides will be useful for the detection of autoimmune diseases such as SLE, scleroderma, primary Sjögren's syn-

drome and polymyositis/dermatomyositis, in which nuclear autoantigens play a role. Said peptides are useful for the development of monoclonal antibodies against these diseases as well as for diagnosing the respective autoimmune diseases, in particular for the detection of autoimmune antibodies in body fluid such as blood, plasma and serum of patients who are suspected of suffering from the autoimmune disease. Again the peptides and antibodies offer the possibility of developing an organic compound with the aid of combinatorial chemistry, which compound is comprised within the scope of the invention.

The recombinant monoclonal antibody described by applicants is reactive with peptide cfc1 but not with the control peptides cfa, cfe, cfq or cf. The commercially available monoclonal antibody AKH1 (Ref. 5), directed against human filaggrin, is not reactive with any of the peptides described herein and is therefore not cross-reactive with an antibody raised against a peptide according to the invention. The polyclonal serum anti-54 kD (Ref. 5), raised against filaggrin, is not reactive with any of the peptides described herein and is therefore not cross-reactive with an antibody reactive with a peptide according to the invention. This suggests that in a normal immune reaction antibodies that are cross-reactive with an antibody raised against a peptide according to the invention, are not necessarily formed.

REFERENCES

- 1) Smolen, J.S., (1996) Autoantibodies in rheumatoid arthritis, in Manual of biological markers of disease (W.J. van Venrooij and R.N. Maini, red.) vol. C Chapter 1.1 pp. 1-18. Kluwer Scientific Publishers, Dordrecht.
- 2) McKinley-Grant, L.J., Idler, W.W., Bernstein, I.A., Parry, D.A.D., Cannizzaro, L., Croce, C.M., Huebner, K., Lessin, S.R. & Steinert, P.M. (1989) Characterization of a cDNA clone encoding human filaggrin and localization of the gene to chromosome region 1q21. Proceedings of the National Academy of Science U.S.A. 86, pp. 4848-4852.
- 3) Gan, S.Q., McBride, O.W., Idler, W.W., Nedialka, M. & Steinert, P.M. (1990) Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry 29, pp. 9432-9440.
- 4) Schellekens, G.A., Lasonder, E., Feijlbrief, M., Koedijk, D.G.A.M., Drijfhout, J.W., Scheffer, A.J., Welling-Wester, S & Welling, G.W. (1994) Identification of the core residues of the epitope of a monoclonal antibody raised against glycoprotein D of herpes simplex virus 1 by screening of a random peptide library. The European Journal of Immunology 24, pp. 3188-3193.
- 5) Hoet, R.M.A., Boerbooms, A.A.Th., Arends, M., Ruiter, D.J., van Venrooij, W.J. (1991) Antiperinuclear factor, a marker autoantibody for rheumatoid arthritis: colocalisation of the perinuclear factor and profilaggrin. Annals of the Rheumatic diseases 50, pp. 611-618.

CLAIMS

1. A peptide derived from an antigen recognized by
5 autoantibodies from patients with rheumatoid arthritis, which
peptide is reactive with autoimmune antibodies from a patient
suffering from rheumatoid arthritis, **characterized** in that
the derived peptide that is reactive with autoimmune anti-
bodies, corresponds to a part of a mRNA molecule coding for
10 the antigen, said part comprising a codon for an arginine
residue, and the arginine residue in the derived peptide,
which is reactive with autoimmune antibodies, is a modified
arginine residue.

2. A peptide according to claim 1, **characterized** in
15 that the modified arginine residue's side chain is a side
chain according to Formula I on the formula sheet, in which

X = NH₂, CH₃, NHCH₃ or N(CH₃)₂;

Y = O, NH, NHCH₃ or N(CH₃)₂;

Z = O, NH or CH₂; and

20 n = 2, 3 or 4, on the condition that when X = NH₂
and Z = NH, Y is not NH.

3. A peptide according to claim 1 or 2, **character-**
ized in that the modified arginine residue is a citrulline
residue.

25 4. A peptide according to one of the preceding
claims, **characterized** in that the peptide is selected from
the group of peptides having the Formula II - X.

5. A peptide according to one of the claims 1 to 3,
characterized in that the peptide is a cyclic peptide.

30 6. A peptide according to claim 5, **characterized** in
that the cyclic peptide is having the Formula XI.

7. A peptide according to one of the preceding
claims, **characterized** in that the peptide is a synthetic
peptide.

35 8. A peptide according to one of the preceding
claims, **characterized** in that the antigen is (pro)filaggrin,
and the peptide is reactive with a rheumatoid arthritis

patient's autoimmune antibodies which are reactive with (pro)filaggrin.

9. A peptide according to one of the claims 1 to 3, **characterized** in that the peptide is obtained by the proteolytic treatment of (pro)filaggrin, separation of peptide fragments formed by proteolysis and subsequent selection on the presence of a modified arginine residue in a peptide which was formed during the proteolytic treatment.

10. An antibody which is cross-reactive with an antibody raised against a peptide according to one of the claims 1 to 9.

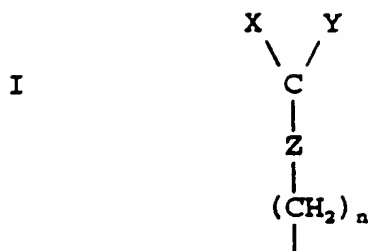
11. An antibody according to claim 10, **characterized** in that the antibody is a monoclonal antibody.

12. An antibody according to claim 10 or 11, **characterized** in that the antibody is obtained by using a peptide according to one of the claims 1 to 9 as an antigen.

13. An antibody according to one of the claims 9 to 12, **characterized** in that it is cross-reactive with the antibody as produced by Escherichia coli TG1 with plasmid RA3, deposited at the Centraalbureau voor Schimmelcultures, at Baarn, the Netherlands under accession number CBS143.96.

14. An organic compound comprising a part that is able to compete with a peptide according to one of the claims 1 to 9 for binding to an antibody which is specific for said peptide, wherein at least said part of the organic compound can be prepared by means of combinatory chemistry.

15. A method for the detection of autoimmune antibodies, **characterized** in that in an immunological test at least one immunologically reactive molecule selected from the group consisting of i) a peptide according to one of the claims 1 to 9; ii) an organic compound according to claim 14; and iii) an antibody according to one of the claims 10 to 13 is used.

FORMULA SHEET

II S H Q E S T X G R S R G R S G R S G S

III S H Q E S T R G X S R G R S G R S G S

IV S H Q E S T R G R S X G R S G R S G S

V S H Q E S T R G R S R G X S G R S G S

VI S H Q E S T R G R S R G R S G X S G S

VII S H Q E S T X G X S R G R S G R S G S

VIII S H Q E S T X G R S X G R S G R S G S

IX S H Q E S T X G R S R G X S G R S G S

X S H Q E S T X G R S R G R S G X S G S

XI H Q C H Q E S T X G R S R G R C G R S G S

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Universiteit van Nijmegen
Biochemie
Postbus 9101
6500 HB Nijmegen
Nederland

*name and address of the party to whom the
viability statement is issued*

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
<p>Name: Universiteit van Nijmegen Biochemie</p> <p>Address: Postbus 9101 6500 HB Nijmegen Nederland</p>	<p>Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:</p> <p style="margin-left: 40px;">CBS 143.96</p> <p>Date of the deposit or of the transfer: ¹</p> <p style="margin-left: 40px;">Wednesday, 13 November 1996</p>
<p>III. VIABILITY STATEMENT</p> <p>The viability of the microorganism identified under II above was tested on Monday, 25 November 1996 ². On that date, the said microorganism was</p> <p><input checked="" type="checkbox"/> ³ viable</p> <p><input type="checkbox"/> ³ no longer viable</p>	

¹ Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY HAS BEEN PERFORMED⁴

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Centraalbureau voor Schimmelfcultures

Address: Oosterstraat 1
P.O. Box 273
3740 AG BAARN
The NetherlandsSignature(s) of person(s) having the power to
represent the International Depositary
Authority or of authorized official(s):drs F.M. van Asma
dr M.C. Agterberg

Date: Monday, 25 November 1996

⁴ Fill in if the information has been requested and if the results of the test were negative.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/47, 16/18, G01N 33/68, 33/564 // C12P 21/00	A3	(11) International Publication Number: WO 98/22503 (43) International Publication Date: 28 May 1998 (28.05.98)
(21) International Application Number: PCT/NL97/00624 (22) International Filing Date: 14 November 1997 (14.11.97) (30) Priority Data: 1004539 15 November 1996 (15.11.96) NL (71) Applicants (for all designated States except US): STICHTING SCHEIKUNDIG ONDERZOEK NEDERLAND [NL/NL]; Laan van Nieuw Oost-Indië 131, NL-2593 BM Den Haag (NL). STICHTING VOOR DE TECHNISCHE WETENSCHAPPEN [NL/NL]; Raadstede 15/19, NL-3431 HA Nieuwegein (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): VAN VENROOIJ, Waltherus, Jacobus, Wilhelmus [NL/NL]; Eleonoraweg 16, NL-6523 MZ Nijmegen (NL). SCHELLEKENS, Gerardus, Antonius [NL/NL]; Zwanenveld 37-03, NL-6538 XV Nijmegen (NL). RAATS, Jozef, Maria, Hendrik [NL/NL]; Van Diemberbroeckstraat 65, NL-6512 BA Nijmegen (NL). HOET, René, Michael, Antonius [NL/NL]; Weezenhof 35 - 40, NL-6536 HB Nijmegen (NL).		(74) Agent: ALTENBURG, Bernardus, Stephanus, Franciscus; Octrooibureau Los en Stigter B.V., Weteringschans 96, NL-1017 XS Amsterdam (NL). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments. In English translation (filed in Dutch).</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13^{bis} separately from the description.</i> <i>Date of receipt by the International Bureau:</i> 5 December 1997 (05.12.97) (88) Date of publication of the international search report: 27 August 1998 (27.08.98)
(54) Title: PEPTIDE DERIVED FROM AN ANTIGEN RECOGNIZED BY AUTOANTIBODIES FROM PATIENTS WITH RHEUMATOID ARTHRITIS, ANTIBODY DIRECTED AGAINST SAID PEPTIDE, A COMBINATORIAL ANTIGEN, AND A METHOD OF DETECTING AUTO-IMMUNE ANTIBODIES (57) Abstract The invention relates to a peptide derived from an antigen recognized by autoantibodies, which peptide is reactive with autoimmune antibodies from a patient suffering from rheumatoid arthritis. The peptide according to the invention possesses a modified arginine residue. The invention also relates to antibodies against the peptide and a method of detecting autoimmune antibodies.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 97/00624

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/47 C07K16/18 G01N33/68 G01N33/564 //C12P21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R. HOET ET AL.: "Antiperinuclear factor, a marker autoantibody for rheumatoid arthritis: colocalisation of the perinuclear factor and profilaggrin." ANNALS OF THE RHEUMATIC DISEASES, vol. 50, no. 9, September 1991, LONDEN, GB, pages 611-618, XP002038024 see page 612, left-hand column, line 14 - line 52 --- -/--	10-13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"S" document member of the same patent family

Date of the actual completion of the international search

12 June 1998

Date of mailing of the international search report

30/06/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 97/00624

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>M. SEBBAG ET AL.: "The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies." THE JOURNAL OF CLINICAL INVESTIGATION, vol. 95, no. 6, June 1995, NEW YORK, NY, VS, pages 2672-2679, XP002038025 see the whole document</p> <p style="text-align: center;">---</p>	10-13
X	<p>M. SIMON ET AL.: "The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies", autoantibodies specific for rheumatoid arthritis." THE JOURNAL OF CLINICAL INVESTIGATION, vol. 92, no. 3, September 1993, NEW YORK, NY, VS, pages 1387-1393, XP002038026 see page 1388, left-hand column, line 21 - line 26</p> <p style="text-align: center;">---</p>	10-13
A	<p>EP 0 511 116 A (CLONATEC S.A.) 28 October 1992 cited in the application see the whole document</p> <p style="text-align: center;">---</p>	1-15
A	<p>S-Q. GAN ET AL.: "Organization, structure and polymorphisms of the human profilaggrin gene." BIOCHEMISTRY, vol. 29, no. 40, 9 October 1990, VS, pages 9432-9440, XP002038027 cited in the application see figures 1,2 see abstract</p> <p style="text-align: center;">---</p>	1-9
A	<p>Y. MISAKI ET AL.: "The 56K autoantigen is identical to human annexin XI." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 6, 11 February 1994, BALTIMORE, MD, VS, pages 4240-4246, XP002038028 see the whole document</p> <p style="text-align: center;">---</p>	1-9
P, O, X	<p>G. SCHELLEKENS ET AL.: "The modified arginine residue citrulline is the major constituent of epitopes recognized by autoantibodies in sera from rheumatoid arthritis patients." ARTHRITIS AND RHEUMATISM, vol. 40, no. 9 suppl., 8 - 12 November 1997, NEW YORK, NY, USA, page S276 XP002067877 see abstract 1471</p> <p style="text-align: center;">---</p>	1-15
	-/--	

INTERNATIONAL SEARCH REPORT

In. .ational Application No

PCT/NL 97/00624

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	FR 2 752 842 A (BIOMÉRIEUX ET AL.) 6 March 1998 see the whole document -----	1, 3, 7-11, 15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 97/00624

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 511116 A	28-10-1992	FR 2675805 A	30-10-1992
		FR 2681600 A	26-03-1993
		CA 2084876 A	27-10-1992
		WO 9219649 A	12-11-1992
		JP 6502187 T	10-03-1994
FR 2752842 A	06-03-1998	WO 9808946 A	05-03-1998